Differential Effect of Spironolactone on the Ulcerogenic and Anti-Inflammatory Activities of Indomethacin (35095)

R. L. ASPINALL
(Introduced by F. J. Saunders)
Department of Endocrinology, Division of Biological Research, G. D. Searle & Co., Chicago, Illinois 60680

Selye has recently reported that intestinal ulcers induced in rats by indomethacin (Indocin) administration can be prevented by concurrent spironolactone (Aldactone) treatment (1–3). Indocin, a potent anti-inflammatory agent in animals (4–6), is employed clinically for the treatment of arthritic conditions in man, but frequent occurrences of gastrointestinal irritation and ulceration has hindered its use (7–9). Selye's data suggest that Aldactone might have clinical utility in preventing this side-effect of Indocin. If however, the anti-inflammatory activity of Indocin was also prevented by Aldactone, there would be no reason to suggest the drug combination.

Therefore, an investigation, utilizing the adjuvant arthritis model in rats (10), was designed to measure the comparative effect of Aldactone on the ulcerogenic and anti-inflammatory activities of Indocin.

Materials and Methods. Male, Sprague-Dawley rats, 160–180 g, were randomly divided into groups of 12 each. Those groups receiving Aldactone were pretreated daily intragastrically for 4 days prior to adjuvant inoculation. The arthritic process was initiated on day 5 (day 1 = first day of Aldactone treatment) by adjuvant inoculation according to the procedure described previously (11). Daily, intragastric, Indocin treatment was started the same day (day 5) and continued separately or together with Aldactone for 14 additional days (through day 19). The arthritic response of the control group, which received saline only, was well advanced by this time, manifesting itself mainly as a swelling of the hind paws.

After sacrifice with CO_2 on day 20, the swelling of the hind paws was measured by mercury volume displacement on a $\Delta V3$ Ugo Basile Plethysmographe (C. H. Stoelting Co., Chicago). The paw volumes of each rat were totaled and the data were analyzed by the Wilcoxon Rank-Sum method (12).

Results. The data from a replicate series of 2 experiments were pooled for analysis (Table I). A fixed dose of 5 mg of Aldactone by itself caused no grossly apparent toxicity and produced no effect on arthritic swelling (Group 2). Treatment with 1 mg of Indocin, however, led to the death of one-third of the rats so treated (Group 3). The deaths occurred during the first week of treatment and gross examination revealed intestinal ulcers, adhesions, and peritonitis. The surviving rats of the same group showed fewer lesions at autopsy. Lesions were rare or absent in the two groups receiving lower doses of Indocin (Groups 4 and 5). Therefore, in this study, mortality was considered to reflect severe ulceration and peritonitis.

The ulcerogenic action of Indocin was almost totally blocked by Aldactone treatment (Group 6); intestinal lesions in this group were not obvious at autopsy. At the same time, however, the anti-inflammatory activity of Indocin was diminished only to a limited degree and only at the upper 2 doses (Groups 6 and 7 compared to Groups 3 and 4, respectively). This depression of the anti-inflammatory activity, though limited, was significant. In spite of this, all doses of Indocin tested, whether with or without Aldactone, produced a highly significant reduction in arthritic swelling.

TABLE I. Effect of 5 mg of Aldactone Daily on the Ulcerogenic and Anti-inflammatory Activities of Various Doses of Indocin in Adjuvant Arthritis.

Group no.	Treatment	Daily dose (mg)	Mortality	Foot volume ± SE[a]	X^b
1	Saline controls	—	0/23	50.1 ± 1.9	—
2	Aldactone	5	0/23	49.5 ± 2.8	0.44 ns
3	Indocin	1	8/24	28.8 ± 1.4	—5.00[c]
4		0.2	1/24	33.5 ± 1.2	—5.27[c]
5		0.04	0/24	43.0 ± 2.2	—2.58[c]
6	Aldactone + Indocin	5 + 1	1/24	38.4 ± 1.6[d]	—4.05[c]
7		5 + 0.2	0/24	40.4 ± 2.1[e]	—3.00[c]
8		5 + 0.04	0/24	42.3 ± 2.4[f]	—2.77[c]

[a] Mean foot volume in plethysmograph meter deflection units ± standard error.

[b] Wilcoxon Rank-Sum values; significance from controls indicated by: ns = not significant; [c] $p < 0.005$, one-tailed.

[d] Significance from Group 3: $p < 0.005$, one-tailed.

[e] Significance from Group 4: $p < 0.01$, one-tailed.

[f] Significance from Group 5: ns, one-tailed.

An additional experiment was conducted with higher doses of Aldactone and Indocin (Table II). Again, Aldactone, even at 20 mg, was well tolerated and without effect on arthritic swelling (Group 2). Indocin by itself at 4 mg (Group 3) was severely ulcerogenic, resulting in the death of the entire group; the 1 mg dose (Group 4) produced similar results as in the previous experiment. The addition of 20 mg of Aldactone/day produced a remarkable sparing action on the ulcerogenic effect of 4 mg of Indocin (Group 5); only one animal died. Five mg of Aldactone, however, was not sufficient to overcome the ulcerogenicity of 4 mg of Indocin (Group 6). The ulcerogenic effect of 1 mg of Indocin was inhibited by 5 mg (Group 7) and 2 mg of Aldactone (Group 8), but 1 mg of Aldactone was not effective (Group 9). The anti-inflammatory activity of Indocin was again affected only to a limited degree by Aldactone (Group 7 compared to Group 4). As before, all doses of Indocin tested, whether with or without Aldactone, produced a highly

TABLE II. Effect of High Doses of Aldactone on the Ulcerogenic and Anti-inflammatory Activities of Varying Doses of Indocin in Adjuvant Arthritis.

Group no.	Treatment	Daily dose (mg)	Mortality	Foot volume ± SE[a]	X^b
1	Saline controls	—	0/12	49.7 ± 2.8	—
2	Aldactone	20	0/12	49.1 ± 3.7	0.17 ns
3	Indocin	4	12/12	—	—
4		1	3/12	34.2 ± 0.9	—3.65[c]
5	Aldactone + Indocin	20 + 4	1/12	28.8 ± 0.6	—4.06[c]
6		5 + 4	11/12	—	—
7		5 + 1	1/12	38.1 ± 1.1[d]	—3.04[c]
8		2 + 1	0/12	35.3 ± 1.4[e]	—3.55[c]
9		1 + 1	3/12	36.3 ± 0.6[f]	—3.34[c]

[a] Mean foot volume in plethysmograph meter deflection units ± standard error.

[b] Wilcoxon Rank-Sum values; significance from controls indicated by: ns = not significant; [c] $p < 0.005$, one-tailed.

[d] Significance from Group 4: $p < 0.01$, one-tailed; [e] ns; [f] $p < 0.05$, one-tailed.

significant reduction in arthritic swelling.

Discussion. Selye has postulated that Aldactone induces in the liver the formation of an enzyme system which is instrumental in the detoxification of Indocin (1–3). In support of this theory is the fact that partial hepatectomy aggrevates the toxic action of Indocin (3); also, that Aldactone stimulates the proliferation of smooth endoplasmic reticulum in hepatocytes (13), which is characteristic in general of inducers of microsomal drug-metabolizing enzymes (14). It has also been shown that the oxidation of pentobarbital by liver microsomes is enhanced by Aldactone (15).

The most significant result of these experiments established that Aldactone, while abolishing the ulcerogenic activity of Indocin, had only a limited effect on Indocin's anti-inflammatory activity. If the postulate, that Aldactone induces Indocin-metabolizing enzyme synthesis, is correct, there are at least two possible explantions for our results which are compatible with this postulate. First, our results could be ascribed to the differential in potency between the ulcerogenic and anti-inflammatory activities of Indocin. Approximately 1 mg of Indocin is required to induce ulcers, whereas the minimal effective anti-inflammatory dose for Indocin in the adjuvant arthritis test is 0.01–0.03 mg (unpulished data). It could be expected, therefore, that the Aldactone-induced enzymes could metabolize Indocin below the threshold concentration necessary to cause ulcers, while leaving a titer sufficient to exert a significant anti-inflammatory effect.

Alternately, the ulcerogenic activity may be due to the Indocin molecule and the anti-inflammatory activity to a metabolite. Aldactone would facilitate the metabolism of the ulcerogenic molecule to the anti-inflammatory metabolite. This theory seems unlikely, however, since the combination resulted in somewhat less anti-inflammatory activity than Indocin by itself.

An alternate postulate to either of the above could suggest that Aldactone has a direct effect on the integrity of the gastrointestinal tract. Aldactone pretreatment could stabilize the intestine against the toxic effect of Indocin, thereby, inhibiting ulcer formation. This postulate, then, would also provide explanation for the retention of the anti-inflammatory activity of Indocin, which would be independent of its toxic effects.

The Aldactone effect on Indocin ulcerogenicity is shared by several other steroids, including SC-11927 (potassium 3-[3-oxo-9α-fluoro-11β-dihydroxy-4-androstene-17α-yl] propanoate), norbolethone, oxandrolone, and ethylestrenol (1). SC-11927, like Aldactone, is a diuretic (16), whereas the remaining three compounds are anabolic agents (17, 18). This group of compounds not only reduces or abolishes the ulcerogenic effect of Indocin, but also induces resistance against the toxic effects of other types of drugs (19–22). The structure–activity relationships are not complete enough to draw firm conclusions as yet, but this effect appears to be unrelated to the primary pharmacological actions of these drugs.

The differential action of Aldactone reported here gives credence to the suggestion that this drug might have an additional clinical application apart from its use as a diuretic. If the ulcerogenic side-effect of Indocin could be lessened or eliminated by the addition of Aldactone without appreciably affecting the anti-inflammatory activity of Indocin, more patients could benefit from Indocin therapy.

Summary. The adjuvant arthritis test was used as a model to measure the effect of Aldactone on the ulcerogenic and anti-inflammatory activities of Indocin. It was demonstrated that Aldactone can abolish the ulcerogenic activity of Indocin while causing only a limited depression of the anti-inflammatory activity. Aldactone, therefore, may be useful in the clinic in reducing the gastrointestinal irritation caused by Indocin treatment.

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